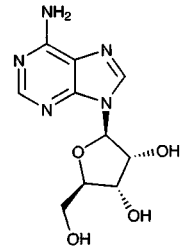
Adenosine



Molecular formula: $C_{10}H_{13}N_5O_4$
Molecular weight: 267.24
CAS Registry No.: 58-61-7
Merck Index: 152

SAMPLE
Matrix: blood
Sample preparation: Plasma, ultrafilter (Amicon) while centrifuging at 1000 g at 20° for 45 min, inject a 50 µL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: 10 × 3 5 µm Chromsep C18
Column: 100 × 4.6 3 µm Microspher C18
Mobile phase: MeCN:50 mM pH 3.7 Na_2HPO_4 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 3.7 nM

OTHER SUBSTANCES
Extracted: inosine

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Huszár,.; Bart,E.; Kollai,M. Isocratic high-performance liquid chromatographic determination of plasma adenosine, *Chromatographia*, **1996**, *42*, 318–322.

SAMPLE
Matrix: blood
Sample preparation: Add 4 mL cold 600 mM perchloric acid to 2 mL blood, let stand at 4° for 10 min, centrifuge. Neutralize a 3 mL aliquot of the supernatant by adding 300 µL 2.5 M potassium carbonate dropwise while stirring, centrifuge. Mix 200 µL of the supernatant with 5′-nucleotidase (from *Crotatus atrox* venom, activity 67 units/mg protein), keep at room temperature, inject 25 µL aliquot.

HPLC VARIABLES
Column: 100 × 8 5 or 10 µm Radial-Pak C18 Resolve cartridge (uncapped) (Waters)
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer:triethylamine 3.5:94.5:2, adjusted to pH 6.9 with phosphoric acid.
Column temperature: 24-26
Flow rate: 2.0
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 10.22
Limit of detection: 10-50 pmole

OTHER SUBSTANCES
Extracted: ATP, ADP, AMP

KEY WORDS
whole blood

REFERENCE
Nishikawa,T.; Suzuki,S.; Ohtani,H.; Shirai,M.; Nomyiama,S.; Kubo,H. Isocratic separation of adenosine 5′-triphosphate and its metabolites by reversed-phase high performance liquid chromatography: end-capped *versus* uncapped packings, *Anal.Sci.*, **1991**, *7*, 241–246.

SAMPLE
Matrix: blood
Sample preparation: Inject 10 μL plasma onto column A and column B in series with mobile phase A, elute with mobile phase A for 4 min then elute the contents of column B onto column C with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES
Column: A 30 × 4.6 44-88 μm Butyl-Toyopearl 650-M (Tosoh); B 30 × 4.6 5 μm Develosil ODS-5 (Nomura); C 250 × 7.6 5 μm Asahipak GS-320H
Mobile phase: A MeCN:2 mM pH 7.4 phosphate buffer 5:95; B MeCN:50 mM bromoacetaldehyde + 150 mM NaCl 15:85, containing 25 mM citrate, pH 5.0
Column temperature: 40° (column C)
Flow rate: A 0.3; B 0.5
Injection volume: 10
Detector: F ex 254 em 400 following post-column heating at 115° in a 15 m × 0.25 mm i.d. reaction coil (Jasco RU-150F unit)

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: adenine
Noninterfering: adenosine triphosphate, adenosine monophosphate, cyclic adenosine monophosphate, adenosine diphosphate

KEY WORDS
plasma; hamster; human; column-switching; post-column reaction; derivatization

REFERENCE
Fujimori,H.; Sasaki,T.; Hibi,K.; Senda,M.; Yoshioka,M. Direct injection of blood samples into a high-performance liquid chromatographic adenine analyser to measure adenine, adenosine, and the adenine nucleotides with fluorescence detection, *J.Chromatogr.*, **1990**, *515*, 363–373.

SAMPLE
Matrix: blood
Sample preparation: 360 μL Blood + 40 μL stopping solution, centrifuge at 14000 g for 1 min. 100 μL Plasma + 10 μL 50% trichloroacetic acid, centrifuge for 5 min. Remove 75 μL of the supernatant and add it to 10 μL 2.3 M KOH, add 50 μL 1 M zinc sulfate, add 100 μL saturated barium hydroxide, vortex for 10 s, centrifuge at 14000 g for 5 min. Remove 100 μL supernatant (pH 5.4) and add it to 10 μL chloroacetaldehyde (45% in water (Fluka)), heat at 80° for 1 h, inject an aliquot. (Stopping solution was 1 mM dialazep, 10μM erythro-9-(2-hydroxy-3-nonyl)adenine, 2 μg/mL indomethacin (final concentrations).)

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was MeOH:10 mM pH 3.5 KH_2PO_4 12:88. B was MeOH:10 mM pH 3.5 KH_2PO_4 50:50. A for 8 min then B for 8 min, re-equilibrate for 4 min.
Flow rate: 1.5
Detector: F ex 280 em 380

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 0.2 pmole

KEY WORDS
plasma; cat; rat; dog; mouse; rabbit; guinea pig; derivatization

REFERENCE
Zhang,Y.; Geiger,J.D.; Lautt,W.W. Improved high-pressure liquid chromatographic-fluorometric assay for measurement of adenosine in plasma, *Am.J.Physiol.*, **1991**, *260*, G658–G664.

SAMPLE
Matrix: blood
Sample preparation: 70 μL Serum + 10 μL 1 mM D-glucosamine.HCl + 20 μL 1 M K_2HPO_4 + 10 μL benzoyl chloride + 25 μL 8 M NaOH, vortex at 2500 vibrations/min for 5 min, add 10 μL 1.4 M phosphoric acid and 100 μL ethyl acetate, vortex at 2500 vibrations/min for 1 min. Remove 25 μL of the ethyl acetate phase and add it to 100 μL MeCN:water 70:30, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Kromasil 100 C18
Column: 250 × 4 5 μm Kromasil 100 C18
Mobile phase: Gradient. MeCN:water from 70:30 to 95:5 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: UV 228 or MS, electrospray, Finnigan MAT, TSQ 700, flow rate 1 μL/min, 2.8 kV, drying gas 140

CHROMATOGRAM
Retention time: 10.9
Internal standard: D-glucosamine (9.7)
Limit of detection: 1-5 pmol

OTHER SUBSTANCES
Extracted: benzyl alcohol, dextrose, mannitol, 2-desoxy-D-glucose, cytidine, myoinositol, sucrose

KEY WORDS
serum; derivatization; fetal bovine serum

REFERENCE
Oehlke,J.; Brudel,M.; Blasig,I.E. Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis, *J.Chromatogr.B*, **1994**, *655*, 105–111.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wave-

length for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 2.697

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate

HPLC VARIABLES
Column: 80 × 4.6 C18 (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 7 KH_2PO_4 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Simultaneous: fludarabine, araA, 2-chloro-2'-arabinofluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine, 2-chloroadenosine, 5'-chloro-5'-deoxyadenosine, 2'-deoxyadenosine

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Structure-activity relationships of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and related analogues: Protein binding, lipophilicity, and retention in reversed-phase LC, *J.Liq.Chromatogr.*, **1995**, *18*, 1123–1135.

SAMPLE
Matrix: perfusate
Sample preparation: Mix 700 μL perfusate (Earle's medium) with 750 μL 24 mM phosphoric acid to adjust pH to 5.4, add 10 μL chloroacetaldehyde, heat at 100° for 40 min, cool in ice, inject a 50 μL aliquot. (Earle's medium contains 116 mM NaCl, 22.6 mM sodium bicarbonate, 5.4 mM KCl, 1.8 mM calcium chloride, 0.8 mM magnesium sulfate, 1.0 mM NaH_2PO_4, 5.5 mM glucose, and 40 g/L dextran (MW 32000-48000), pH 8.5-8.6. Prepare chloroacetaldehyde by refluxing chloroacetaldehyde dimethyl acetal:1.5 M sulfuric acid 5:1 for 30 min, distil, collect the fraction boiling at 85-95°, use undiluted.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm NewGuard RP-18
Column: 100 × 4.6 3 μm ODS-Hypersil C18
Mobile phase: Gradient. MeOH:10 mM pH 6.7 $(NH_4)H_2PO_4$ from 0:100 to 1:99 over 1 min, to 3:97 over 1 min, to 6:94 over 1 min, to 10:90 over 1 min, to 15:85 over 1 min, to 23:77 over 1 min, to 35:65 over 1 min, to 50:50 over 1 min, to 60:40 over 1 min, to 80:20 over 0.5 min, maintain at 80:20 over 0.5 min, return to initial conditions over 0.2 min, re-equilibrate for 8.8 min.
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 275 em 415

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 2 nM

KEY WORDS
derivatization

REFERENCE
Slegel,P.; Kitagawa,H.; Maguire,M.H. Determination of adenosine in fetal perfusates of human placental cotyledons using fluorescence derivatization and reversed-phase high-performance liquid chromatography, *Anal.Biochem.*, **1988**, *171*, 124–134.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer + 100 μL 600 nM vidarabine in water, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)
Column: 150 × 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0.)
Flow rate: 1
Detector: F ex 305 em 420

CHROMATOGRAM
Retention time: 22
Internal standard: vidarabine (17)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS
derivatization

REFERENCE

Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE

Matrix: perfusate
Sample preparation: Direct injection of a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 octyl Spherisorb S5-ODS
Mobile phase: MeCN:100 mM KH_2PO_4 13:87
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 262

REFERENCE

Michael-Baruch,E.; Shiri,Y.; Cohen,S. Alkali halide-assisted penetration of neostigmine across excised human skin: A combination of structured water disruption and a Donnan-like effect, *J.Pharm.Sci.*, **1994**, *83*, 1071–1076.

SAMPLE

Matrix: perfusate, tissue
Sample preparation: Homogenize (glass to glass) tissue with 1 mL ice-cold 400 mM perchloric acid, centrifuge at 10000 g for 10 min, dilute the supernatant 10-fold with Krebs solution. Mix 1 mL (?) perfusate (Krebs solution) or tissue homogenate with 25 (perfusate) or 50 (tissue) μL chloroacetaldehyde, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0°.)

HPLC VARIABLES

Column: 5 μm Radial-Pak C18
Mobile phase: Gradient. A was 100 mM pH 6.0 phosphate buffer. B was MeOH:100 mM pH 6.0 phosphate buffer 25:75. A:B from 100:0 to 0:100 over 15 min (Waters concave curve 8), maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 2
Detector: F ex 300 em 420

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.5-1 pmole

OTHER SUBSTANCES

Extracted: adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS

derivatization; guinea pig; vas deferens

REFERENCE

Levitt,B.; Head,R.J.; Westfall,D.P. High-performance liquid chromatographic-fluorometric detection of adenosine and adenine nucleotides: Application to endogenous content and electrically induced release of adenyl purines in guinea pig vas deferens, *Anal.Biochem.*, **1984**, *137*, 93–100.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS2
Mobile phase: MeOH:200 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 1.5
Detector: UV 260

OTHER SUBSTANCES
Simultaneous: ADP, AMP, ATP

REFERENCE
Ziganshin,A.U.; Ziganshina,L.E.; King,B.F.; Pintor,J.; Burnstock,G. Effects of P2-purinoceptor antagonists on degradation of adenine nucleotides by ecto-mucleotidases in folliculated oocytes of Xenopus laevis, *Biochem.Pharmacol.*, **1996**, *51*, 897–901.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 4
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 0.4

OTHER SUBSTANCES
Simultaneous: deoxyinosine, didanosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: deoxyinosine, didanosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

SAMPLE

Matrix: tissue

Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)

Column: 150 × 4.6 3 μm LC-18-T (Supelco)

Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for 12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 1.2

Injection volume: 20

Detector: UV 266

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Extracted: adenosine triphosphate, ascorbic acid

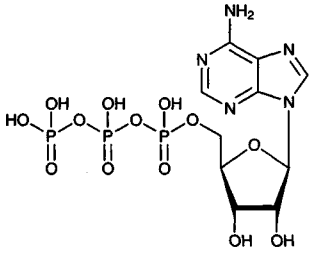
KEY WORDS

rat; heart

REFERENCE

Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

Adenosine triphosphate



Molecular formula: $C_{10}H_{16}N_5O_{13}P_3$
Molecular weight: 507.18
CAS Registry No.: 56-65-5
Merck Index: 154

SAMPLE

Matrix: blood

Sample preparation: Isolate mononuclear cells from 10 mL blood by a standard step-gradient density centrifugation procedure. Wash once with PBS, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash the pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, neutralize with 10 M KOH, bring to pH 7 with 1 M KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject a 50-2000 μL aliquot of the supernatant. (PBS was 8.1 g NaCl, 0.22 g KCl, 1.14 g $NaHPO_4$ (sic), 0.27 g KH_2PO_4 in 1 L water, pH 7.4.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil 10 SAX

Mobile phase: Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)

Flow rate: 3

Injection volume: 50-2000

Detector: UV 262

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: ara-CTP (cytarabine triphosphate), Fara-ATP (fludarabine triphosphate), CTP, UTP, GTP

KEY WORDS

mononuclear cells

REFERENCE

Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE

Matrix: blood

Sample preparation: Dilute whole blood 10-100 fold with 320 mM sucrose, inject 10 μL of the diluted solution onto column A and column B in series with mobile phase A, elute with mobile phase A for 4 min then elute the contents of column B onto column C with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES

Column: A 30 × 4.6 44-88 μm Butyl-Toyopearl 650-M (Tosoh); B 10 × 4 3 μm Hitachi gel 3013-N; C 50 × 4.6 3 μm Hitachi gel 3013-N

Mobile phase: A MeCN:water 15:85; B MeCN:50 mM bromoacetaldehyde + 150 mM NaCl 15:85, containing 25 mM citrate, pH 4.0
Column temperature: 45° (column C)
Flow rate: A 0.3; B 0.3
Injection volume: 10
Detector: F ex 254 em 400 following post-column heating at 115° in a 15 m × 0.25 mm i.d. reaction coil (Jasco RU-150F unit)

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: adenosine monophosphate, cyclic adenosine monophosphate, adenosine diphosphate
Noninterfering: adenine, adenosine

KEY WORDS
whole blood; hamster; rat; human; column-switching; post-column reaction; derivatization

REFERENCE
Fujimori,H.; Sasaki,T.; Hibi,K.; Senda,M.; Yoshioka,M. Direct injection of blood samples into a high-performance liquid chromatographic adenine analyser to measure adenine, adenosine, and the adenine nucleotides with fluorescence detection, *J.Chromatogr.*, **1990**, *515*, 363–373.

SAMPLE
Matrix: cell cultures
Sample preparation: Freeze in liquid nitrogen. 2 mL Frozen cell suspension + 1 mL cold 3 M perchloric acid, sonicate at 0° for 30 s, neutralize with 2 M KOH containing 300 mM 4-morpholinepropanesulfonic acid (MOPS), centrifuge at 3000 g for 10 min, inject an aliquot.

HPLC VARIABLES
Guard column: Valco guard column containing C18 pellicular material
Column: 150 × 4.6 Excellopak ODS C18 (R.E. Gourley)
Mobile phase: MeOH:100 mM pH 6.0 potassium phosphate 3.75:96.25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Extracted: NADP+, NAD+, adenosine diphosphate, adenosine monophosphate

KEY WORDS
hepatocyte

REFERENCE
Litt,M.R.; Potter,J.J.; Mezey,E.; Mitchell,M.C. Analysis of pyridine dinucleotides in cultured rat hepatocytes by high-performance liquid chromatography, *Anal.Biochem.*, **1989**, *179*, 34–36.

SAMPLE
Matrix: cell suspensions
Sample preparation: 1 mL Cell suspension + 500 μL ice-cold MeCN + 500 μL water, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES
Column: Partisil-10 SAX
Mobile phase: Gradient. A was 30 mM pH 4.8 ammonium phosphate. B was MeCN:700 mM pH 4.6 ammonium phosphate 10:90. A:B 100:0 for 5 min then a convex gradient to 75:25 over 10 min then a convex gradient to 0:100 over 15 min, stay at 0:100 for 15 min
Flow rate: 1.7
Detector: UV 254

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Extracted: metabolites, didanosine

REFERENCE
Mukherji,E.; Au,J.L.-S.; Mathes,L.E. Differential antiviral activities and intracellular metabolism of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine in human cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1573–1579.

SAMPLE
Matrix: cells
Sample preparation: Separate cells from 10 mL whole blood, wash with phosphate-buffered saline, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, adjust pH to 7.0 with 10 KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10 SAX
Mobile phase: Gradient. 5 mM pH 2.8 $(NH_4)H_2PO_4$:750 mM pH 3.5 $(NH_4)H_2PO_4$ from 70:30 to 0:100 over 30 min (concave gradient Waters curve 9) (At the start of each day purge with 20 mL 2 M $(NH_4)H_2PO_4$, inject 100 μL 100 mM disodium EDTA under initial gradient conditions.)
Flow rate: 3
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: CTP, UTP, GTP, cytarabine triphosphate, fludarabine triphosphate

KEY WORDS
human cells

REFERENCE
Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 800 μL chloroform:MeOH 2:1, agitate vigorously for 1 min, centrifuge, inject a 5 μL aliquot of the upper water/MeOH layer.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 55:45 containing 9 mM KH_2PO_4, pH 5.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Extracted: ciprofibrate, metabolites

KEY WORDS
rat; liver

REFERENCE
Bronfman,M.; Amigo,L.; Morales,M.N. Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters, *Biochem.J.*, **1986**, *239*, 781–784.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer + 100 μL 600 nM vidarabine in water, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)
Column: 150 × 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0.)
Flow rate: 1
Detector: F ex 305 em 420

CHROMATOGRAM
Retention time: 3.5
Internal standard: vidarabine (17)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: adenosine, adenosine diphosphate, adenosine monophosphate

KEY WORDS
derivatization

REFERENCE
Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE

Matrix: perfusate, tissue

Sample preparation: Homogenize (glass to glass) tissue with 1 mL ice-cold 400 mM perchloric acid, centrifuge at 10000 g for 10 min, dilute the supernatant 10-fold with Krebs solution. Mix 1 mL (?) perfusate (Krebs solution) or tissue homogenate with 25 (perfusate) or 50 (tissue) μL chloroacetaldehyde, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0°.)

HPLC VARIABLES

Column: 5 μm Radial-Pak C18

Mobile phase: Gradient. A was 100 mM pH 6.0 phosphate buffer. B was MeOH:100 mM pH 6.0 phosphate buffer 25:75. A:B from 100:0 to 0:100 over 15 min (Waters concave curve 8), maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 2

Detector: F ex 300 em 420

CHROMATOGRAM

Retention time: 12

Limit of detection: 1 pmole

OTHER SUBSTANCES

Extracted: adenosine, adenosine diphosphate, adenosine monophosphate

KEY WORDS

derivatization; guinea pig; vas deferens

REFERENCE

Levitt,B.; Head,R.J.; Westfall,D.P. High-performance liquid chromatographic-fluorometric detection of adenosine and adenine nucleotides: Application to endogenous content and electrically induced release of adenyl purines in guinea pig vas deferens, *Anal.Biochem.*, **1984**, *137*, 93–100.

SAMPLE

Matrix: solutions

Sample preparation: 10 μL Solution + 200 μL buffer + 10 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in buffer + 40 μL 50 mM dansylethylenediamine in DMSO, mix, let stand in the dark at 27° for 18 h, inject a 10 μL aliquot. (Buffer was 100 mM pH 7.5 1-methylimidazole buffer. Synthesis of dansylethylenediamine is as follows. Add a solution of dansyl chloride in DMF to a 10% molar excess of ethylenediamine in DMF, evaporate to dryness, dissolve the residue in pH 10 carbonate buffer, purify by TLC, extract with MeOH.)

HPLC VARIABLES

Column: 250 × 4.6 Finepak ODP-50 octadecyl-bonded polyvinyl alcohol gel (Asahikasei, Tokyo)

Mobile phase: Gradient. A was MeCN:10 mM pH 10.3 phosphate buffer 12:88. B was MeCN:10 mM pH 10.3 phosphate buffer 22:78. C was MeCN:10 mM pH 10.3 phosphate buffer 40:60. A:B:C 100:0:0 for 10 min, to 0:100:0 over 18 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 10 min.

Column temperature: 40

Flow rate: 0.6

Injection volume: 10
Detector: F ex 270 em 546

CHROMATOGRAM
Retention time: 11
Limit of detection: 4.7-20.3 pmole

OTHER SUBSTANCES
Simultaneous: adenosine diphosphate, adenosine monophosphate, cytidine monophosphate, guanosine diphosphate, guanosine monophosphate, guanosine triphosphate, uridine monophosphate

KEY WORDS
derivatization

REFERENCE
Sonoki,S.; Sanda,A.; Hisamatsu,S. Simultaneous determination of mono-, di-, and trinucleotides by high-performance liquid chromatography using *N*-(dansyl)ethylenediamine as a fluorescent derivatizing reagent, *J.Liq.Chromatogr.*, **1994**, *17*, 1057–1064.

SAMPLE
Matrix: tissue
Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)
Column: 150 × 4.6 3 μm LC-18-T (Supelco)
Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for 12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 45

OTHER SUBSTANCES
Extracted: adenosine, ascorbic acid

KEY WORDS
rat; heart

REFERENCE
Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 1 g frozen powdered tissue with 9 mL 2.5% perchloric acid. Neutralize a 200 μL aliquot with 2 M KOH containing 200 mM K_2HPO_4, centrifuge at 4° at 9500 g for 1 min, inject an aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm Hypersil
Column: 125 × 4.6 5 μm Hypersil 5ODS
Mobile phase: Gradient. A was 100 mM pH 6.0 potassium phosphate buffer containing 8 mM tetra-n-butylammonium hydrogen sulfate and 15 mM chloroacetaldehyde. B was MeCN:water 75:25. A:B from 97:3 to 65:35 over 6 min, return to initial conditions over 1 min.
Flow rate: 1.6
Detector: F ex 230 em 430 following post-column reaction. The column effluent flowed through a knitted 10 m × 0.5 mm ID PTFE coil at 100° to the detector.

CHROMATOGRAM

Retention time: 8.5
Limit of detection: 10 pmole

OTHER SUBSTANCES

Extracted: adenosine diphosphate, adenosine monophosphate

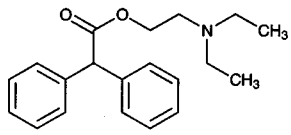
KEY WORDS

post-column reaction; mouse; liver

REFERENCE

Stratford,M.R.L.; Dennis,M.F. Determination of adenine nucleotides by fluorescence detection using high-performance liquid chromatography and post-column derivatization with chloroacetaldehyde, *J.Chromatogr.B*, **1994**, *662*, 15–20.

Adiphenine



Molecular formula: $C_{20}H_{25}NO_2$
Molecular weight: 311.42
CAS Registry No.: 64-95-9, 50-42-0 (HCl), 6113-04-8 (methyl bromide)
Merck Index: 160

SAMPLE
Matrix: solutions

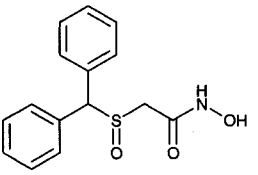
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 250 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 260

CHROMATOGRAM
Retention time: k' 2.3

OTHER SUBSTANCES
Simultaneous: diphenylacetic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr. Rel.Technol.*, **1998**, *21*, 1347–1357.

Adrafinil



Molecular formula: $C_{15}H_{15}NO_3S$
Molecular weight: 289.36
CAS Registry No.: 63547-13-7
Merck Index: 168

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.833

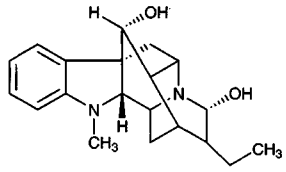
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ajmaline



Molecular formula: $C_{20}H_{26}N_2O_2$
Molecular weight: 326.44
CAS Registry No.: 4360-12-7
Merck Index: 194

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 246

CHROMATOGRAM

Retention time: 4.37

Limit of detection: <120 ng/mL

KEY WORDS

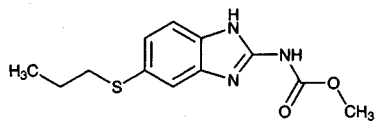
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Albendazole



Molecular formula: $C_{12}H_{15}N_3O_2S$
Molecular weight: 265.34
CAS Registry No.: 54965-21-8
Merck Index: 211
Lednicer No.: 2 353

SAMPLE

Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid
Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: oxfendazole, thiabendazole, cambendazole, mebendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS

plasma; sheep

REFERENCE

Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

SAMPLE

Matrix: blood
Sample preparation: 200-1000 μL Plasma + 150 ng proguanil hydrochloride (in water) + 200-1000 μL MeCN, vortex for 5 s, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 1 mL water, add 10 mL dichloromethane, vortex for 10 s, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 10-50 μL aliquot.

HPLC VARIABLES

Guard column: 10 μm CM Guard-Pak (Waters)
Column: 100 × 8 10 μm μBondapak phenyl
Mobile phase: MeOH:MeCN:1% triethylamine 10:20:70
Flow rate: 2.5
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.9
Internal standard: proguanil (5.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Hoaksey,P.E.; Awadzi,K.; Ward,S.A.; Coventry,P.A.; Orme,M.L'E.; Edwards,G. Rapid and sensitive method for the determination of albendazole and albendazole sulphoxide in biological fluids, *J.Chromatogr.*, **1991**, *566*, 244–249.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 200 μL 500 mM ammonium hydroxide (to adjust pH to 11) + 200 mg NaCl + 5 mL distilled diethyl ether, roll for 15 min, remove 4 mL supernatant, repeat extraction, remove 5 mL supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 60 μL MeOH, sonicate for 2 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 5 Nucleosil 5C18
Mobile phase: MeCN:1% acetic acid 43:57
Flow rate: 0.9
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 1.6
Internal standard: albendazole

OTHER SUBSTANCES
Extracted: febantel, fenbendazole, oxfendazole

KEY WORDS
plasma; albendazole is IS; oxfendazole sulfone; sheep

REFERENCE
Landuyt,J.; Debackere,M.; Delbeke,F.; McKellar,Q. A high performance liquid chromatographic method for the determination of febantel and its major metabolites in lamb plasma, *Biomed.Chromatogr.*, **1993**, *7*, 78–81.

SAMPLE
Matrix: blood, CSF
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 17 mM KH_2PO_4 adjusted to pH 5.5 with 800 mM NaOH. 2 mL Plasma or CSF + 100 μL 5 μg/mL mebendazole in MeOH + 2 mL 10 mM KH_2PO_4 adjusted to pH 7.4 with 800 mM NaOH, vortex for 30 s, add to the SPE cartridge, wash with 20 mL 10 mM KH_2PO_4 adjusted to pH 7.4 with 800 mM NaOH, wash with 1 mL MeOH:water 20:80, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS C18
Mobile phase: MeOH:50 mM pH 5.7 phosphate buffer 70:30
Flow rate: 0.8
Injection volume: 20
Detector: UV 295

CHROMATOGRAM
Retention time: 11.6
Internal standard: mebendazole (8.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Hurtado,M.; Medina,M.T.; Sotelo,J.; Jung,H. Sensitive high-performance liquid chromatographic assay for albendazole and its main metabolite albendazole sulphoxide in plasma and cerebrospinal fluid, *J.Chromatogr.*, **1989**, *494*, 403–407.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize lung tissue in 5 volumes 100 mM pH 6.0 Na_2HPO_4 and centrifuge at 1500 g for 15 min. 500 μL Serum or 1 mL lung tissue homogenate supernatant + 100 μL 3 μg/mL mebendazole in MeOH:DMSO 90:10 + 1 mL 100 mM potassium carbonate + 4 mL dichloromethane, mix on an Eberbach shaker for 10 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeOH:MeCN:70 mM monochloroacetic acid 27:18:55
Flow rate: 1.2
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Internal standard: mebendazole

KEY WORDS
serum; lung; mouse

REFERENCE
Bartlett,M.S.; Edlind,T.D.; Lee,C.H.; Dean,R.; Queener,S.F.; Shaw,M.M.; Smith,J.W. Albendazole inhibits Pneumocystis carinii proliferation in inoculated immunosuppressed mice, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1834–1837.

SAMPLE
Matrix: blood, urine
Sample preparation: 100-200 μL Whole blood, plasma, or urine + 1 mL 200 ng/mL mebendazole in ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject.

HPLC VARIABLES
Column: 250 × 4.6 normal phase 5 μm Partisil
Mobile phase: Hexane:EtOH 89:11
Flow rate: 2
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 4.7
Internal standard: mebendazole (7.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; sheep; normal phase; pharmacokinetics

REFERENCE
Galtier,P.; Alvinerie,M.; Steimer,J.L.; Francheteau,P.; Plusquellec,Y.; Houin,G. Simultaneous pharmacokinetic modeling of a drug and two metabolites: application to albendazole in sheep, *J.Pharm.Sci.*, **1991**, *80*, 3–10.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM
Retention time: 17.777

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve sample in MeOH containing 10% formic acid, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 19:81, pH 3.9 (Buffer was prepared by dissolving 6.6 g dibasic ammonium phosphate in 1 L water and adjusting to pH 3.9 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: fenbendazole, niclosamide, oxyclozanide

KEY WORDS
tablets; powder; liquid formulations

REFERENCE
van Tonder,E.C.; de Villiers,M.M.; Handford,J.S.; Malan,C.E.P.; Du Preez,J.L. Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations, *J.Chromatogr.A*, **1996**, *729*, 267–272.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Place 1.5 mL microsomal incubation in a boiling water bath for 2 min, add to a Sep-Pak C18 SPE cartridge, add mebendazole to the SPE cartridge, wash with water, elute with MeOH, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil C18
Mobile phase: Gradient. MeCN:0.5% acetic acid 35:65 for 5 min, 70:30 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1 for 5 min, 1.5 for 5 min, re-equilibrate at 1
Detector: UV 292

CHROMATOGRAM
Retention time: 8.2
Internal standard: mebendazole (7.3)
Limit of detection: 20 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; intestine; SPE

REFERENCE
Villaverde,C.; Alvarez,A.I.; Redondo,P.; Voces,J.; del Estal,J.L.; Prieto,J.G. Small intestinal sulphoxidation of albendazole, *Xenobiotica*, **1995**, *25*, 433–441.

SAMPLE
Matrix: milk

Sample preparation: 1 mL Milk + 100 μL 400 mM NaOH, extract with 8 mL ethyl acetate, vortex at high speed for 3 s, centrifuge at 4000 g for 2 min. Remove a 6 mL aliquot of the clear supernatant, add 2 mL water, vortex for 10 s, centrifuge at 1000 g for 30 s. Remove the organic layer and evaporate it to dryness under nitrogen at 40°. Reconstitute the residue in 500 μL mobile phase, filter (0.2 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:10 mM phosphoric acid 20:80 containing 5 mM tetrabutylammonium hydrogen sulfate
Column temperature: 50
Flow rate: 1
Injection volume: 100
Detector: UV 292

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 5 ng/mL
Limit of quantitation: 1.6 ng

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: amoxicillin, ampicillin, chlortetracycline, cloxacillin, febantel, febendazole, febendazole hydroxide, febendazole sulfone, gentamicin, kanamycin, mebendazole, neomycin, oxacillin, oxfendazole, oxibendazole, oxytetracycline, penicillin G, penicillin V, streptomycin, tetracycline, thiabendazole, thiabendazole hydroxide, triclabendazole

KEY WORDS

milk; cow; pharmacokinetics

REFERENCE

Fletouris,D.J.; Boytsoglou,N.A.; Psomas,I.E.; Mantis,A.I. Trace analysis of albendazole and its sulphoxide and sulphone metabolites in milk by liquid chromatography, *J.Chromatogr.B*, **1996**, *687*, 427–435.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 45:55 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 305

OTHER SUBSTANCES

Also analyzed: epirizole, prochlorperazine

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: tissue

Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Retention time: 9.5
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES

Extracted: thiabendazole, oxfendazole, fenbendazole

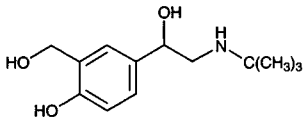
KEY WORDS

matrix solid-phase dispersion; liver

REFERENCE

Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

Albuterol



Molecular formula: $C_{13}H_{21}NO_3$
Molecular weight: 239.31
CAS Registry No.: 18559-94-9, 51022-70-9 (sulfate)
Merck Index: 217
Lednicer No.: 2 43

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase , mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: F ex 222 em 300

CHROMATOGRAM

Retention time: 20.6
Internal standard: albuterol

OTHER SUBSTANCES

Extracted: atenolol
Noninterfering: chlorthalidone, xipamide

KEY WORDS

plasma; albuterol is IS

REFERENCE

Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE

Matrix: solutions
Sample preparation: Dilute 800 μL solution to 10 mL with water, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: RP-18
Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:buffer 4:96 for 6 min, to 9:91 (step gradient). (Buffer was 40 mM NaH_2PO_4 containing 5.74 mM triethylamine, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 265

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Mälkki-Laine,L.; Hartikainen,E. Electrokinetic behaviour of salbutamol and its decomposition products and determination of salbutamol by micellar electrokinetic capillary chromatography, *J.Chromatogr.A*, **1996**, *724*, 297–306.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 CSP-4 (Prepare as follows. Add a solution of 1.07 g L-valyl-L-valyl-L-valine isopropylester (Bunseki Kagaku 1079, 28, 125) in 30 mL dry dioxane (Caution! Dioxane is a carcinogen!) dropwise to a mixture of 2.2 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 20 mL dry dioxane stirred at 0°, add 3 g anhydrous sodium carbonate at room temperature, stir, filter, evaporate to give a colorless solid. Dissolve 8.3 g of this solid in 30 mL dry dioxane, add 2 g N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, add 1.5 g anhydrous sodium carbonate, reflux with stirring for 40 h, filter, add 3 g dried 10 μm LiChrosorb Si 100, reflux with slow stirring for 10 h, cool, filter. Wash the solid with dioxane, MeOH, and diethyl ether, dry under reduced pressure (J.Chromatogr. 1984, 292, 427).)
Mobile phase: Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 60:37.5:3.75:0.25
Detector: UV

CHROMATOGRAM
Retention time: k' 5.84 (first enantiomer)

KEY WORDS
chiral; α = 1.06

REFERENCE
Oi,N.; Kitahara,H.; Matsushita,Y.; Kisu,N. Enantiomer separation by gas and high-performance liquid chromatography with tripeptide derivatives as chiral stationary phases, *J.Chromatogr.A*, **1996**, *722*, 229–232.

Aldesleukin

Molecular formula: $C_{690}H_{1115}N_{177}O_{203}S_6$
Molecular weight: 15600
CAS Registry No.: 110942-02-4

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL sample with 225 μL MeOH and 125 μL chloroform. Add 125 μL 100 mM HCl and 125 μL chloroform. Mix thoroughly and centrifuge at 7500 g for 5 min. Inject a 100 μL aliquot of the water/MeOH layer. (Caution! Chloroform is a carcinogen!)

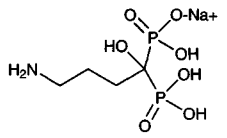
HPLC VARIABLES

Column: Phenomenex W–Porex C4
Mobile phase: MeCN:water containing 100 mM sodium perchlorate and 10 mM perchloric acid 52.5:47.5
Column temperature: 30
Injection volume: 100
Detector: UV 205

REFERENCE

Koppenhagen,F.J.; Visser,A.J.W.G.; Herron,J.N.; Storm,G.; Crommelin,D.J.A. Interaction of recombinant interleukin-2 with liposomal bilayers, *J.Pharm.Sci.*, **1998**, *87*, 707–714.

Alendronate sodium



Molecular formula: $C_4H_{12}NNaO_7P_2$
Molecular weight: 271.08
CAS Registry No.: 121268-17-5 (trihydrate), 66376-36-1 (free acid)
Merck Index: 228

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Condition an Analytichem 3 mL 500 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 µL 2.5 M calcium chloride, vortex, add 50 µL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 µL 1 M HCl, add 5 mL water, precipitate by adding 50 µL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 µL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 µL of the eluate and add it to 250 µL 100 mM pH 9.1 sodium borate buffer, vortex, add 10 µL 50 mM KCN, add 10 µL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 µL aliquot. Plasma. Condition an Analytichem 3 mL 500 mg diethylamine SPE cartridge with 3 mL water. 1 mL Plasma + 1 mL 10% trichloroacetic acid, vortex, centrifuge at 5300 g for 10 min. Remove the supernatant and add it to 50 µL 100 mM sodium pyrophosphate in water, add 50 µL 2.5 M calcium chloride, vortex, add 50 µL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 µL 1 M HCl, add 5 mL water, precipitate by adding 50 µL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 µL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 µL of the eluate and add it to 250 µL 100 mM pH 9.1 sodium borate buffer, vortex, add 10 µL 50 mM KCN, add 10 µL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 µL aliquot. (Buffer was 50 mM sodium citrate:50 mM pH 8.5 sodium phosphate buffer 1:1.)

HPLC VARIABLES

Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 µm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: MeOH:buffer 40:60 (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 8.5 with 10 mM NaOH.)
Flow rate: 1
Injection volume: 100
Detector: F ex 420 em 490

CHROMATOGRAM

Retention time: 5
Limit of quantitation: 5 ng/mL (urine)

KEY WORDS

plasma; derivatization; SPE

REFERENCE

Kline,W.F.; Matuszewski,B.K.; Bayne,W.F. Determination of 4-amino-1-hydroxybutane-1,1-bisphosphonic acid in urine by automated pre-column derivatization with 2,3-naphthalene dicarboxaldehyde and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *534*, 139–149.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 μL 1.25 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 100 μL 1 M HCl, add 5 mL water, precipitate by adding 100 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 800 μL 200 mM acetic acid, add 400 μL 10 mM EDTA, add 400 μL 200 mM sodium acetate, add 3 mL water, add to the SPE cartridge, elute with 1 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 1 mg/mL N-acetyl-D-penicillamine in MeOH, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, inject a 50 μL aliquot. Plasma. Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 1 mL Plasma + 3 mL water + 250 μL 10% trichloroacetic acid, vortex, add three more 250 μL portions of 10% trichloroacetic acid with vortexing each time, centrifuge at 5300 g for 10 min. Remove the supernatant and add it to 200 μL 22 mM sodium pyrophosphate in water, add 50 μL 2.5 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 100 μL 1 M HCl, add 5 mL water, precipitate by adding 100 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 800 μL 200 mM acetic acid, add 400 μL 10 mM EDTA, add 400 μL 200 mM sodium acetate, add 3 mL water, add to the SPE cartridge, elute with 1 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 1 mg/mL N-acetyl-D-penicillamine in MeOH, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, inject a 50 μL aliquot. (Buffer was 200 mM sodium citrate: 200 mM pH 8.5 Na_2HPO_4 buffer 1:1.)

HPLC VARIABLES

Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 μm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: Gradient. MeCN:buffer at 15:85 for 10 min, to 32.5:67.5 over 5 min, return to initial conditions over 5 min, re-equilibrate for 10 min. (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 6.3 with 85% phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: F ex 436 em 440 (cut-off filter)

CHROMATOGRAM

Retention time: 7
Limit of detection: 0.2 ng/mL (urine)
Limit of quantitation: 5 ng/mL (plasma), 1 ng/mL (urine)

KEY WORDS

plasma; derivatization; SPE

REFERENCE

Kline,W.F.; Matuszewski,B.K. Improved determination of the bisphosphonate alendronate in human plasma and urine by automated precolumn derivatization and high-performance liquid chromatography with fluorescence and electrochemical detection, *J.Chromatogr.*, **1992**, *583*, 183–193.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections to a concentration of 25 μg/mL with 100 mM sodium citrate. Shake 1 tablet or the contents of 1 capsule with 50 mL 100 mM sodium citrate for 30 min, sonicate for 5 min, make up to 100 mL with 100 mM sodium citrate, dilute to 25 μg/mL, centrifuge at 2000 rpm for 10 min. Mix 5 mL supernatant or diluted injection with 5 mL 100 mM pH 9.0 sodium borate, add 4 mL 500 μg/mL 9-fluorenylmethyl chloroformate in MeCN, vortex for 30 s, let stand for 30 min, add 25 mL dichloromethane, shake for 30-60 s, let stand for 5 min, centrifuge at 1000 rpm for 5 min, inject a 50 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:MeOH:buffer 20:5:75 (Prepare buffer by dissolving 14.7 g sodium citrate dihydrate and 8.7 g K_2HPO_4 in 900 mL water, adjust pH to 8.0 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 266

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
derivatization; injections; capsules; tablets

REFERENCE
De Marco,J.D.; Biffar,S.E.; Reed,D.G.; Brooks,M.A. The determination of 4-amino-1-hydroxybutane-1,1-diphosphonic acid monosodium salt trihydrate in pharmaceutical dosage forms by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1719–1727.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Simultaneous: clodronate, etidronate, neridronate, olpadronate, pamidronate

KEY WORDS
derivatization; complexation; injections; tablets

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE
Matrix: formulations
Sample preparation: Stir tablets containing 40 mg alendronate in 100 mL water for 30 min, filter (0.22 μm) an aliquot, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 6 μm IC-Pak HR polymethacrylate quaternary ammonium anion-exchange (Waters)
Mobile phase: 6 mM nitric acid
Column temperature: 25
Flow rate: 0.5
Injection volume: 50
Detector: RI

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 400 ng/mL

KEY WORDS
tablets

REFERENCE
Han,Y.-H.R.; Qin,X.-Z. Determination of alendronate sodium by ion chromatography with refractive index detection, *J.Chromatogr.A*, **1996**, *719*, 345–352.

SAMPLE
Matrix: urine
Sample preparation: Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 μL 2.5 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 μL 1 M HCl, add 5 mL water, precipitate by adding 50 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 μL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 50 mM KCN, add 10 μL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 μL aliquot. (Buffer was 50 mM sodium citrate:50 mM pH 8.5 sodium phosphate buffer 1:1.)

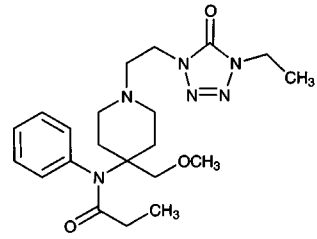
HPLC VARIABLES
Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 μm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: MeOH:buffer 40:60 (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 8.5 with 10 mM NaOH.)
Flow rate: 1
Injection volume: 100
Detector: E, LC-4B (Bioanalytical Systems), LC-17A ED flow cell with glassy carbon electrode +0.65 V, RE-4 Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.4
Limit of quantitation: 2.5 ng/mL

KEY WORDS
derivatization; SPE

REFERENCE
Kline,W.F.; Matuszewski,B.K. Improved determination of the bisphosphonate alendronate in human plasma and urine by automated precolumn derivatization and high-performance liquid chromatography with fluorescence and electrochemical detection, *J.Chromatogr.*, **1992**, *583*, 183–193.

Alfentanil



Molecular formula: $C_{21}H_{32}N_6O_3$
Molecular weight: 416.52
CAS Registry No.: 71195-58-9, 69049-06-5 (HCl), 70879-28-6 (HCl monohydrate)
Merck Index: 236
Lednicer No.: 3 118

SAMPLE

Matrix: blood, urine
Sample preparation: 50 µL Plasma or urine + 50 µL 4 M NaOH + 100 µL MeCN + 500 µL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 µm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 5.78
Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: fentanyl, sufentanil

KEY WORDS

plasma

REFERENCE

Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

SAMPLE

Matrix: cell cultures
Sample preparation: Freeze a 400 µL aliquot of the hepatocyte culture in hexane/dry ice, add an equal volume of DMSO, thaw, vortex, sonicate for 5 s, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 5 µm Hypersil C18
Mobile phase: Gradient. A was 100 mM pH 7.0 ammonium acetate. B was MeCN:MeOH:THF:1 M pH 7.0 ammonium acetate 30:20:40:10. A:B from 100:0 to 40:60 over 40 min.
Flow rate: 1
Injection volume: 200
Detector: UV 230

CHROMATOGRAM

Retention time: 40

OTHER SUBSTANCES

Extracted: metabolites

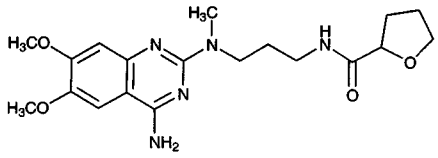
KEY WORDS

hepatocyte cultures; rat; dog

REFERENCE

Lavrijsen,K.; Van Houdt,J.; Meuldermans,W.; Knaeps,F.; Hendrickx,J.; Lauwers,W.; Hurkmans,R.; Heykants,J. Metabolism of alfentanil by isolated hepatocytes of rat and dog, *Xenobiotica*, **1988**, *18*, 183–197.

Alfuzosin



Molecular formula: $C_{19}H_{27}N_5O_4$
Molecular weight: 389.45
CAS Registry No.: 81403-80-7, 81403-68-1 (HCl)
Merck Index: 237
Lednicer No.: 4 149

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 2 mg/mL IS in MeOH, vortex, add 1 mL 100 mM NaOH, add 7 mL dichloromethane:diethyl ether (nonstabilized) 3:4, stir for 25 min. Centrifuge at 1000 g at 4° for 5 min, remove the organic layer, evaporate it to dryness under a stream of nitrogen at 30°. Dissolve the residue in 80 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm Chiral-AGP (ChromTech)
Mobile phase: MeCN:buffer 6:94 (Buffer was 50 mM KH_2PO_4 adjusted to pH 7.4 with 1 M NaOH.)
Flow rate: 0.9
Injection volume: 20
Detector: F ex 265 em 400

CHROMATOGRAM

Retention time: 4 (R), 6 (S)
Limit of detection: 1 ng/mL

KEY WORDS

chiral; plasma

REFERENCE

Krstulovic,A.M.; Vende,J.L. Improved performance of the second generation α 1-AGP columns: applications to the routine assay of plasma levels of alfuzosin hydrochloride, *Chirality*, **1989**, *1*, 243–245.

SAMPLE

Matrix: blood
Sample preparation: Dilute 100 μL urine to 1 mL with water. 1 mL PLasma, whole blood, or diluted urine + 10 μL 5 μg/mL IS in water + 1 mL 100 mM NaOH + 7 mL diethyl ether, shake for 30 min, centrifuge at 4° at 1000 g for 5 min. Remove 6.5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 870 μL MeCN:20 mM pH 2.5 KH_2PO_4 10:90, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:20 mM pH 2.5 KH_2PO_4 60:40
Flow rate: 1
Injection volume: 500
Detector: F ex 334 em 378

CHROMATOGRAM

Retention time: 4.4
Internal standard: N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)amino]propyl]-N-methyltetrahydro-2H-pyran-2-carboxamide hydrochloride (5.6)
Limit of detection: 0.5 ng/mL

KEY WORDS
plasma; whole blood

REFERENCE
Guinebault,P.; Broquaire,M.; Colafranceschi,C.; Thénot,J.P. High-performance liquid chromatographic determination of alfuzosin in biological fluids with fluorimetric detection and large-volume injection, *J.Chromatogr.*, **1986**, *353*, 361–369.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2 μg/mL IS in MeOH + 1 mL 100 mM NaOH + 7 mL dichloromethane:diethyl ether 3:4, shake for 25 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 80 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm chiral-AGP (ChromTech)
Mobile phase: MeCN:buffer 6:94, pH 7.4 (Buffer was 0.667 g KH_2PO_4, 4.587 g $K_2HPO_4.3H_2O$, and 8.060 g tetrabutylammonium bromide in 1 L water.)
Flow rate: 0.9
Injection volume: 20
Detector: F ex 265 em 400

CHROMATOGRAM
Retention time: 5.32 (R), 6.58 (S)
Internal standard: N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]-4,5-dihydro-2-furancarboxamide (9.75)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: furosemide, zolpidem
Noninterfering: betaxolol, captopril, diazepam, enalapril, metoprolol, piroxicam, propranolol, warfarin

KEY WORDS
chiral; plasma; pharmacokinetics

REFERENCE
Rouchouse,A.; Manoha,M.; Durand,A.; Thenot,J.P. Direct high-performance liquid chromatographic determination of the enantiomers of alfuzosin in plasma on a second-generation α 1-acid glycoprotein chiral stationary phase, *J.Chromatogr.*, **1990**, *506*, 601–610.

SAMPLE
Matrix: blood
Sample preparation: Inject 50 μL plasma on to column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A on to column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 50 × 4.6 10 μm LiChrosorb C18; B 20 × 4.6 30 μm Pellicular-CN (Merck) + 250 × 4.6 5 μm Spherisorb S5W cyanopropyl
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:50 mM pH 2.5 phosphate buffer 38:2:60
Flow rate: 1
Injection volume: 50
Detector: F ex 265 em 400

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 1 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Carlucci,G.; Di Giuseppe,E.; Mazzeo,P. Determination of alfuzosin in human plasma by high-performance liquid chromatography with column-switching, *J.Liq.Chromatogr.*, **1994**, *17*, 3989–3997.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

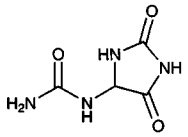
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 244

CHROMATOGRAM
Retention time: 10.37

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Allantoin



Molecular formula: $C_4H_6N_4O_3$
Molecular weight: 158.12
CAS Registry No.: 97-59-6
Merck Index: 255

SAMPLE

Matrix: blood
Sample preparation: Cool 1 mL plasma in an ice bath, add 100 μL 1.1 mM allopurinol in water, add 100 μL 4 M perchloric acid, vortex for 1 min, cool in an ice bath for 10 min, centrifuge at 10000 g for 5 min. Remove the supernatant and adjust the pH to 6-7 with ice-cold 4 M KOH using bromothymol blue as an indicator, let stand for 10 min, centrifuge at 2000 g for 10 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-5
Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid 20:80. A:B at 100:0 for 5 min, to 98:2 over 2 min, to 0:100 over 23 min, re-equilibrate at 100:0 for 20 min.
Flow rate: 0.8
Detector: UV 205

CHROMATOGRAM

Retention time: 3
Internal standard: allopurinol (16)
Limit of detection: 0.06 nmole

OTHER SUBSTANCES

Extracted: metabolites, uric acid, xanthine, hypoxanthine

KEY WORDS

plasma; sheep

REFERENCE

Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE

Matrix: blood
Sample preparation: 300 μL Plasma + 600 μL MeCN, mix vigorously, centrifuge at 2500 g for 10 min, remove the supernatant, add 600 μL MeCN:water 2:1 to the residue, mix vigorously, centrifuge. Combine the supernatants and add them to the SPE cartridge, recover the eluate, elute with 2 mL MeCN:1 mM phosphoric acid 50:50. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL 100 mM NaOH, heat on a boiling water bath for 20 min, cool, add 600 μL 1.5 mM 2,4-dinitrophenylhydrazine in 2.5 M HCl, heat at 50° for 1 h, centrifuge at 10000 g for 30 min, inject a 50 μL aliquot of the supernatant. (Prepare the SPE cartridges by making a slurry of AG1-X8 (chloride form) strong ion-exchange resin (Bio-Rad) in water, let stand for 30 min, add 0.5 mL of the slurry to a 65 × 5 polypropylene column fitted with a 20 μm polyethylene frit, add another frit to the top, wash the column with water until no chloride ions are eluted, equilibrate with 3 mL MeCN:1 mM phosphoric acid 50:50. Test for the presence of chloride ions by adding 1 mL eluate to 300 μL 300 mM silver nitrate solution, a white precipitate indicates the presence of chloride ions.

Regenerate columns by washing with 5 mL 1 M HCl, wash with 20 mL water until no chloride ions are detected.)

HPLC VARIABLES

Guard column: Reversed-phase guard column (Whatman)
Column: 100 × 4.6 Partisphere C18 (Whatman)
Mobile phase: Gradient. A was MeCN:100 mM pH 6.0 KH_2PO_4 5:95. B was MeCN:100 mM pH 6.0 KH_2PO_4 50:50. A:B 100:0 for 1 min, to 84:16 over 20 min, to 0:100 over 9 min, re-equilibrate at the initial conditions for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 360

CHROMATOGRAM

Retention time: 16.6
Limit of quantitation: 4 μM

KEY WORDS

plasma; SPE; derivatization

REFERENCE

Lagendijk,J.; Ubbink,J.B.; Vermaak,W.J.H. The determination of allantoin, a possible indicator of oxidant status, in human plasma, *J.Chromatogr.Sci.*, **1995**, *33*, 186–193.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 3 mL 10% trichloroacetic acid (cooled to 2-4°) to 3 mL plasma (cooled to 0-4°) or urine acidified to pH ≤3 with 1 M sulfuric acid and diluted 20 or 40 times with water, centrifuge at 4000 g at 4° for 15 min. Add a 500 μL aliquot of the supernatant to 50 μL 0.04% thymol blue pH indicator. If the reaction mixture color is orange (pH about 1.3) add 50 μL 600 mM NaOH, if the color fails to change to blue add more 600 mM NaOH. When the color changes to blue (pH 9.3-11) heat the reaction mixture at 85° for 60 min. Add 200 μL derivatizing solution (the color becomes orange-yellow), continue the heating for 20 min, filter (0.2 μm), inject an aliquot of the filtrate. (Prepare the derivatizing solution by dissolving 100 mg 2,4-dinitrophenylhydrazine in 100 mL 2 M HCl and by filtering through a membrane filter.)

HPLC VARIABLES

Guard column: 10 × 6 30-40 μm C18 pellicular material (Waters)
Column: two 250 × 4.6 4 μm Nova-Pak C18 columns in series
Mobile phase: Gradient. A was 2.5 mM ammonium dihydrogen phosphate adjusted to pH 3.5 with 10% H_3PO_4. B was MeCN:2.5 mM pH 3.5 ammonium dihydrogen phosphate 20:80. A:B 100:0 for 7.7 min, to 0.8:99.2 in 0.6 min, from 0.8:99.2 to 0.4:99.6 in 8.7 min, from 0.4:99.6 to 100:0 in 31.5 min, maintain at 100:0 for 11.5 min.
Flow rate: 0.9 for 17.1 min, 1 for 3.9 min, 1.2 for 39 min
Injection volume: 10
Detector: UV 205 for 9 min, UV 254 for 16 min, then UV 360

CHROMATOGRAM

Retention time: 37.6
Limit of detection: 4 pmol

OTHER SUBSTANCES

Extracted: uric acid, hypoxanthine, xanthine

KEY WORDS

plasma; sheep; urine

REFERENCE

Czauderna,M.; Kowalczyk,J. Simultaneous measurement of allantoin, uric acid, xanthine and hypoxanthine in blood by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *704*, 89–98.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Adjust pH to < 3 with 1 M sulfuric acid, dilute so that the concentration of allantoin was 0.03-0.32 mM. 500 μL + 50 μL indicator + 100 μL 600 mM NaOH, if the color did not change to blue (pH > 9.2) add 600 mM NaOH in 50 μL increments, heat at 85° for 1 h, add 200 μL reagent, heat at 85° for 20 min, cool, centrifuge at 35000 g for 15 min, inject a 10 μL aliquot of the supernatant. Plasma. 2 mL Plasma + 2 mL 10% trichloroacetic acid, centrifuge at 35000 g for 20 min. 500 μL supernatant + 50 μL indicator + 100 μL 600 mM NaOH, if the color did not change to blue (pH > 9.2) add 600 mM NaOH in 50 μL increments, heat at 85° for 1 h, add 200 μL reagent, heat at 85° for 20 min, cool, centrifuge at 35000 g for 15 min, inject a 10 μL aliquot of the supernatant. (Indicator was 600 mM NaOH and 0.04% thymol blue in water, filter (Whatman No. 1 paper). Reagent was 1 g/L 2,4-dinitrophenylhydrazine in 2 M HCl, filter (Whatman No. 1 paper).)

HPLC VARIABLES

Guard column: 25 × 2 30-40 μm pellicular reversed-phase C18

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was MeCN:10 mM acetic acid, pH adjusted to 6.1 with ammonia 15:85. B was MeCN. A:B from 100:0 to 82:18 over 3 min (Waters concave no. 7) to 18:82 over 2 min (Waters concave no. 7), stay at 18:82 for 3 min, to 100:0 over 1 min (linear), re-equilibrate at 100:0 for 15 min.

Flow rate: 1

Injection volume: 10

Detector: UV 360

CHROMATOGRAM

Retention time: 3, 7.3 (syn- and anti-derivatization products)

Limit of quantitation: 5000 nM

KEY WORDS

plasma; derivatization

REFERENCE

Chen,X.B.; Kyle,D.J.; Orskov,E.R. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1993**, *617*, 241–247.

SAMPLE

Matrix: urine

Sample preparation: Adjust pH to < 3 with sulfuric acid, dilute 1:20 with 100 mM $(NH_4)H_2PO_4$, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-5

Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 4.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 4.0 with phosphoric acid 20:80. A:B from 100:0 to 0:100 over 30 min, stay at 0:100 for 10 min, re-equilibrate at 100:0 for 20 min.

Flow rate: 0.8

Injection volume: 20

Detector: UV 205

CHROMATOGRAM

Retention time: 3

Limit of detection: 0.06 nmole

OTHER SUBSTANCES
Extracted: metabolites, uric acid, hypoxanthine

KEY WORDS
sheep

REFERENCE
Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm), dilute 10-fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: 10 mM pH 4.0 potassium phosphate buffer
Column temperature: 25
Flow rate: 0.5
Injection volume: 20
Detector: UV 218

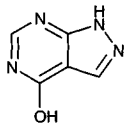
CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 0.2 mg/mL

OTHER SUBSTANCES
Extracted: creatinine

KEY WORDS
sheep

REFERENCE
Resines,J.A.; Díez,M.T.; Arín,M.J. Statistical evaluation of agreement between HPLC and colorimetric methods for analysis of allantoin in ruminants' urine, *J.Liq.Chromatogr.*, **1993**, *16*, 2853–2859.

Allopurinol



Molecular formula: $C_5H_4N_4O$
Molecular weight: 136.11
CAS Registry No.: 315-30-0
Merck Index: 287
Lednicer No.: 1 152, 269

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 75 μL 10 μg/mL acetaminophen in water, mix, inject a 20 μL aliquot directly.

HPLC VARIABLES

Guard column: 23 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 50 mM pH 6.0 phosphate buffer
Flow rate: 2.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 7.2
Internal standard: acetaminophen (18)
Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Extracted: uric acid, oxipurinol

KEY WORDS

plasma; renew guard column after 50-70 injections

REFERENCE

Nissen,P. Simultaneous determination of allopurinol, oxipurinol and uric acid in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 382–386.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 102.7 μg/mL floxuridine in water, vortex 10 s, add 200 μL water containing 30% w/v trichloroacetic acid and 30% w/v perchloric acid, vortex 10 s, place in an ice bath for 2 min, centrifuge at 3000 g for 20 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 5μm Hypersil-ODS
Mobile phase: 20 mM Na_2HPO_4 adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: floxuridine (7)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: oxipurinol

KEY WORDS
plasma

REFERENCE
Hung,C.T.; Zoest,A.R.; Perrier,D.G. Analysis of allopurinol and oxipurinol in plasma by reversed phase HPLC, *J.Liq.Chromatogr.*, **1986**, *9*, 2471–2483.

SAMPLE
Matrix: blood
Sample preparation: Cool 1 mL plasma in an ice bath, add 100 μL 4 M perchloric acid, vortex for 1 min, cool in an ice bath for 10 min, centrifuge at 10000 g for 5 min. Remove the supernatant and adjust the pH to 6-7 with ice-cold 4 M KOH using bromothymol blue as an indicator, let stand for 10 min, centrifuge at 2000 g for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-5
Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid 20:80. A:B at 100:0 for 5 min, to 98:2 over 2 min, to 0:100 over 23 min, re-equilibrate at 100:0 for 20 min.
Flow rate: 0.8
Detector: UV 205

CHROMATOGRAM
Retention time: 16
Internal standard: allopurinol

OTHER SUBSTANCES
Extracted: allantoin, uric acid, xanthine, hypoxanthine

KEY WORDS
plasma; sheep; allopurinol is IS

REFERENCE
Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute plasma 1:2 (plasma) or 1:20 (urine) with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.1 5 μm SAS-Hypersil
Mobile phase: pH 7.0 Buffer containing 190 mL 100 mM citric acid, 810 mL 200 mM disodium phosphate, and 2 L water.
Flow rate: 2
Injection volume: 20
Detector: UV 252

CHROMATOGRAM
Retention time: 10
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: oxipurinol

Noninterfering: aminophylline, aspirin, 8-azaguanine, azathioprine, benzbromarone, caffeine, cotrimoxazole, cytarabine, diazepam, dihydralazine, dipyridamole, fluorouracil, guanine, hypoxanthine, 6-mercaptopurine, methotrexate, procarbazine, propranolol, spironolactone, sulfinpyrazone, 6-thioguanine, uric acid, xanthine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Breithaupt,H.; Goebel,G. Determination of allopurinol and oxipurinol in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *226*, 237–242.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. 0.5 mL Urine + 5 mL 20 mM pH 8.0 sodium phosphate buffer, mix, inject a 10-50 μL aliquot. Plasma. 0.5 mL Plasma + 0.4 mL water + 0.1 mL 20% perchloric acid, mix, centrifuge at 1300 g at 4° for 10 min. Mix 0.5 mL of the supernatant with 0.5 mL 200 mM disodium phosphate, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 300 × 3.9 8-10 μm μBondapak C18
Mobile phase: 4 mM pH 6.0 sodium phosphate buffer
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM

Retention time: 18.8
Limit of detection: 100-300 ng/mL

OTHER SUBSTANCES

Extracted: orotidine, orotic acid, uric acid, creatinine, hypoxanthine, xanthine, oxipurinol

KEY WORDS

plasma

REFERENCE

Miyazaki,H.; Matsunaga,Y.; Yoshida,K.; Arakawa,S.; Hashimoto,M. Simultaneous determination of plasma and urinary uric acid, xanthine, hypoxanthine, allopurinol, oxipurinol, orotic acid, orotidine and creatinine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 75–85.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 12.5 μM 9-methylxanthine + 150 μL 12% trichloroacetic acid, centrifuge, inject an aliquot. Urine. Inject directly.

HPLC VARIABLES

Guard column: 50 × 4.6 3 μm Hypersil ODS
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM KH_2PO_4 adjusted to pH 3.65 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: 9-methylxanthine
Limit of detection: 150 ng/mL

OTHER SUBSTANCES
Simultaneous: oxipurinol, xanthine, hypoxanthine

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bory,C.; Baltassat,P.; Gonnet,C. Simultaneous determination of allopurinol, oxipurinol, hypoxanthine and xanthine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *307*, 469–471.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount containing ca. 50 mg allopurinol, add 10 mL 100 mM NaOH, shake 10 min, dilute to 50 mL, filter (0.45 μm) discarding first 10 mL of filtrate. 4 mL Filtrate + 2 mL IS solution, make up to 200 mL with mobile phase, mix, inject a 10-20 μL aliquot. (Carry out sample preparation and injection without delay. IS solution was 50 mg hypoxanthine, add 10 mL 100 mM NaOH, shake 10 min, dilute to 50 mL. Prepare fresh daily.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak
Mobile phase: 50 mM Ammonium dihydrogen phosphate (For overnight storage flush system with water for at least 20 min and with MeOH for 20 min.)
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: hypoxanthine (7)

KEY WORDS
tablets

REFERENCE
Shostak,D. Liquid chromatographic determination of allopurinol in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1121–1122.